Studies on antibiotic aerosols for inhalation in cystic fibrosis

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6 Dry powder inhalation of colistin sulfomethate in healthy volunteers: a pilot study

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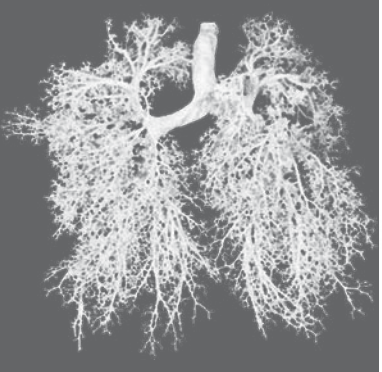

Int J Pharm 2007; 335:41-45.

Summary

Pulmonary administration of the antimicrobial drugs colistimethate sodium (colistin sulfomethate) and tobramycin has been shown to be effective in slowing down pulmonary deterioration in cystic fibrosis (CF) patients. Both drugs are administered by liquid nebulisation, a technique known to have disadvantages. Dry powder inhalation may be an attractive alternative. We investigated inhalation of colistimethate sodium dry powder using a newly developed Twincer® device in healthy volunteers.

Eight healthy volunteers inhaled a single dose of 25 mg colistimethate sodium dry powder each, using the Twincer® inhaler. The median diameter (X_{50}) of the dry powder was 1.6 µm (X_{10} = 0.7 µm, X_{90} = 3.1 µm), measured by laser diffraction technique. Pulmonary function tests were performed before, 5 and 30 min after inhalation. Serum samples were drawn at t = 15 min, 45 min, 1.5 h, 2.5 h, 3.5 h, 5.5 h, 7.5 h and 24 h after inhalation.

The colistimethate sodium dry powder inhaler was well tolerated: no clinically relevant effect on FEV_1 was observed nor did the volunteers experience adverse effects.

Dry powder inhalation of colistimethate sodium using the Twincer® inhaler is well tolerated by healthy volunteers. A pilot study in cystic fibrosis patients is therefore considered safe in developing a dry powder inhalation of colistimethate sodium for everyday CF treatment.

Introduction

Cystic fibrosis (CF) is a hereditary disease affecting multiple organs. Anomalies in the respiratory system are prominent, resulting in main disease symptoms. Mortality due to respiratory failure is common. Due to a genetic defect of the transmembrane conductance regulator (CFTR) gene on chromosome 7, chloride secretion in the lung is disturbed resulting in highly viscous sputum. As a consequence mucoid clearance is reduced, allowing foreign material (bacteria) to remain behind. Chronic infection with bacteria, especially *Pseudomonas aeruginosa,* induces inflammation and fibrosis of the lung, resulting in deterioration of lung capacity.
Pulmonary administration of antimicrobial drugs, such as the anti-pseudomonal drugs colistimethate sodium (colistin sulfomethate) and tobramycin, has been shown to be effective in interfering with and slowing down of pulmonary deterioration (Touw *et al.,* 1995; Mukhopadhyay *et al.,* 1996; Hodson *et al.,* 2002). Both drugs are administered by liquid nebulisation, using either an ultrasonic or jet nebuliser technique. Although nebulisation of drugs may have advantages for specific patient groups, routine home use of nebulisers in CF patients has some drawbacks. Most prominent is the amount of time necessary for complete nebulisation of a dose. Including drug preparation and cleaning afterwards, nebulisation of a dose takes approximately 15–30 min, depending on the nebuliser used. Adherence of CF patients to nebulised therapy has been shown to be poor (Abbott *et al.,* 1994; Conway *et al.,* 1996; Burrows *et al.,* 2002). Furthermore, individual breathing pattern, physico-chemical properties of (combinations of) inhalation liquids and nebuliser performance play a role in the nebulisation process, all of influence on the treatment effect.

The best approach to reduce the influence of the breathing pattern of an individual patient on drug deposition of an inhalation dose is to design inhalation devices that depend less strongly on patient characteristics. Adaptive aerosol delivery (AAD) is an example, as is the AKITA® inhalation system. Dry powder inhalers (DPI's) have a main advantage in time gain in relation to drug administration, compared to liquid nebulisation. Furthermore, the size of a DPI is more practical and convenient in everyday use, there is no need for an electrical power source to administer a dose and a DPI device is generally lower in costs because of a relatively simple design in which there is no need for electronic parts. Because of these advantages, an increase in patient adherence is to be expected. However, patient characteristics too play a role in successfully inhaling a dry powder drug. The currently commercially available 'breath controlled' dry powder inhalers all require a minimal inspiratory flow rate in order to reach a sufficient deagglomeration of the dry powder dose. Few inhalers have been designed in order to diminish the influence of the patient's ability to produce an adequate inspiratory flow rate on the inhalation manoeuvre. The dependency on inspiratory flow rate may be reduced by the use of additional energy for dispersion of the dose (Exubera® inhaler) or by a larger fine particle fraction (Novolizer® inhaler) to compensate for a shift in deposition of the dose to the upper airways at higher inspiratory flow rate. Another option is to design an inhaler which combines

a high dispersion effectiveness with a relatively high internal resistance with which a maximal fine particle fraction can be generated even at a low inspiratory effort. This technique is applied in the current study by using the Twincer® inhaler. In CF patients, inspiratory flow is generally not influenced by the pulmonary disease process (Sarinas *et al.,* 1998) and therefore dry powder inhalation may be useful.

The optimal dose of nebulised colistimethate sodium in CF is not known; most adult patients are treated with 2 million units (approximately 160 mg) of colistimethate sodium twice daily based on empiric experience. Administration of colistimethate sodium as a dry powder requires a calculation of an equivalent dose to liquid nebulisation. The efficiency of the dry powder inhaler is therefore of main importance. The aim is to administer an equivalent pulmonary dose using the least possible number of inhalations. However, to achieve this, an inhalation of colistimethate sodium dry powder will have to consist of milligrams instead of micrograms. Commercially available dry powder inhalers are suited for doses in the microgram to 1 mg range. De Boer *et al.* (2006) described the development of a newly dry powder inhalation device (Twincer®), intended for disposable use, which can deliver doses effectively in the 1–25 mg range. *In vitro* results of this new DPI device are promising. We investigated the inhalation of colistimethate sodium dry powder using the Twincer® device in healthy volunteers.

Materials and methods

Study population

Eight healthy volunteers were recruited by advertisement. Inclusion criteria were age 18–40 years, no history of chronic pulmonary disease, not pregnant or breast-feeding, non-smoking and an informed consent. The volunteers' demographic data are listed in Table 1. The study was performed according to the Helsinki declaration and was approved of by the medical ethical review board of the hospital. Volunteers were fully informed by the investigators and a written informed consent was obtained from every volunteer.

Table 1 Volunteer demographic data

Subject	Sex (M/F)	Age (years)	Height (cm)	Weight (kg)
V1	F	33	163	64
V2	M	31	175	85
V3	F	22	176	72
V4	F	20	171	71
V5	F	21	155	54
V6	F	31	158	91
V7	F	23	173	64
V8	M	22	180	75
Mean (S.D.)		25.4 (5.3)	168.9 (9.1)	72.0 (11.9)

Study drug

Colistimethate sodium (Ph. Eur. quality, sterile) was obtained from Alpharma, Copenhagen, Denmark. One milligram contained approximately 13,200 units. The particle size of the raw material had to be reduced in order to obtain particles in the desired (aerodynamic) size range of 1–5 µm. Particle size reduction was performed in the hospital pharmacy under Good Manufacturing Practice conditions using a jet mill (50 AS, Alpine, Augsburg, Germany). A mass median diameter (X_{50}) of 1.6 µm was obtained (X_{10} = 0.7 µm, X_{90} = 3.1 µm), determined by laser diffraction technique. The fine particle fraction (<5 µm) was 43.8% at 34 l/min (1 kPa), 48.0% at 48 l/min (2 kPa) and 50.6% at 67 l/min (4 kPa) from a dose of 2×12.5 mg colistimethate sodium. The dry powder container was protected from air humidity as much as possible, in order to prevent agglomeration of hygroscopic colistimethate sodium powder particles between milling and use. Each volunteer inhaled a colistimethate sodium dry powder mixture containing colistimethate sodium 83.3% (25 mg) and lactose 16.7% with a machined prototype of the Twincer® (University of Groningen, Groningen, The Netherlands; De Boer *et al.*, 2006) on day 1. The Twincer® inhaler devices were manually filled with 12.5 mg of micronised colistimethate sodium in each of the two dose compartments. Approximately 2.5 mg of lactose (150–200 µm, Pharmatose 100 M, DMV International, Veghel, The Netherlands) was added to each compartment, to act as a sweeper. Preparation of the inhaler was done shortly before each administration.

Prior to inhalation the volunteers received inhalation instructions. This was done using an empty inhaler connected to an electronic inspiratory flow measurement device (University of Groningen, Groningen, The Netherlands). Inspiratory flow rate (l/min) and inhalation time (s) were registered for each volunteer, after the inhalation manoeuvre could be performed consistently. Participants were instructed to perform a deep and powerful inhalation during 2–3 s and to hold their breath during a few seconds. Inhalation of the colistimethate sodium dry powder dose was done immediately after instruction. After inhalation, the volunteers were observed and asked for adverse effects. After inhalation, the inhaler was tested for the amount of drug remaining in the device. The inhaler was rinsed with water and the remaining colistimethate sodium was determined using a modification of the protein assay described by Lowry (Lowry *et al.,* 1951). The actual inhaled dose of colistimethate sodium was calculated by subtracting the amount of drug remaining in the device after inhalation from the total dose weighed into the inhaler. The next day (day 2), each volunteer swallowed 80 mg colistimethate sodium dissolved in 3 ml NaCl 0.9% on an empty stomach, in order to investigate gastro-intestinal absorption of colistin.

Pulmonary function tests

Pulmonary function tests were performed before, 5 and 30 min after inhalation. Forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC) were measured using a calibrated Masterlab pneumotachograph (Jaeger, Würzburg, Germany). The volunteers received lung function

test instructions. Measured pulmonary function parameters were normalized to the reference values proposed by the European Community for Coal and Steel (Quanjer *et al.,* 1993). Change (percentage) in lung function is relative to baseline, and not a percentage fall of predicted. A reduction in FEV_1 of 10% or more was considered to be clinically significant.

Blood sampling and serum analysis

Venous blood samples were collected after inhalation of colistimethate sodium. Samples were taken at 15 min, 45 min, 1.5 h, 2.5 h, 3.5 h, 5.5 h, 7.5 h and 24 h after inhalation. Furthermore, after ingestion of colistimethate sodium on day 2, blood was collected from the volunteers at t=1 and 3 h. Analysis of serum samples was performed using a modification of the method described by Le Brun (Le Brun *et al.,* 2000). A calibration curve in the concentration range of 24–120 µg/l was used. The method of analysis was shown to be linear over a concentration range of 24–724 µg/l. A lower limit of quantification (LLQ) of 11 µg/l was calculated by the method described by Kucharczyk (1993).

Pharmacokinetic analysis

Serum concentration results were studied using the MW\Pharm software (version 3.58, Medi-Ware, Groningen, tThe Netherlands; Proost and Meijer 1992). A population pharmacokinetic model was made from data of all volunteers using the Bayesian iterative two stage method (KINPOP module). Subsequently individual pharmacokinetic parameters were calculated, using the obtained population model.

Results

Pulmonary function

No clinically relevant effect on lung function was observed from inhalation of 2×12.5 mg colistimethate sodium. Pulmonary function test data are listed in Table 2. None of the volunteers experienced objective or subjective adverse effects due to inhalation of the colistin dry powder. Mean peak inspiratory flow rate through the empty inhaler device was 76.9 l/min (range 68.9–86.9); mean inhalation time was 2.3 s (range 1.7–2.6). All participants were able to hold their breath for at least 3–4 s.

Pharmacokinetic data

Colistimethate sodium inhalation pharmacokinetics could be described using a one-compartment open model with drug input from a peripheral compartment without lag time and drug elimination from the central compartment. An overview of individual fitted serum concentration time curves of the volunteers is displayed in Fig. 1. Pharmacokinetic parameters are listed in Table 3. The actual dose of colistimethate sodium inhaled by each subject was used

Table 2 Volunteer pulmonary function data

Subject	$FEV_{1,t=0min}$	$\Delta FEV_{1,t=5min}$	$\Delta FEV_{1,t=30min}$	$FVC_{t=0min}$	$\Delta FVC_{t=5min}$	$\Delta FVC_{t=30min}$
V1	3.72 (124)	-4.8	-1.1	4.60 (133)	-2.6	-2.8
V2	4.15 (100)	-1.7	-1.0	5.37 (109)	+1.3	-2.2
V3	4.15 (111)	-1.9	-1.0	4.26 (100)	-0.7	-1.4
V4	4.00 (113)	+2.5	-1.0	4.97 (123)	-0.4	-3.2
V5	3.43 (118)	+2.3	+3.8	3.92 (118)	+2.8	+1.5
V6	3.07 (107)	-1.3	-2.9	3.42 (104)	+0.9	-1.5
V7	3.71 (103)	-3.2	-0.8	3.96 (96)	-4.6	-0.5
V8	5.46 (121)	-1.1	-0.2	6.44 (120)	+0.9	+0.2
Mean	3.96 (112)			4.62 (113)		
SD	0.71 (9)			0.96 (13)		

FEV_1: forced expiratory volume in 1 s (l), % predicted. ΔFEV1: change in FEV1 compared to baseline, in %. FVC: forced vital capacity (l), % predicted. ΔFVC: change in FVC compared to baseline, in %. $t = 0$ min: baseline; prior to inhalation of colistin. $t = 5$ min: at $t = 5$ min after inhalation of colistin. $t = 30$ min: at t=30 min after inhalation of colistin. S.D.: standard deviation.

in the pharmacokinetic calculations. Colistin serum concentrations after oral ingestion of 80 mg of liquid colistimethate sodium on day 2 remained below the LLQ of the analytical assay method used and were therefore not detectable. It may be concluded that no contribution of gastro-intestinally absorbed colistimethate sodium to serum concentrations after inhalation is to be expected.

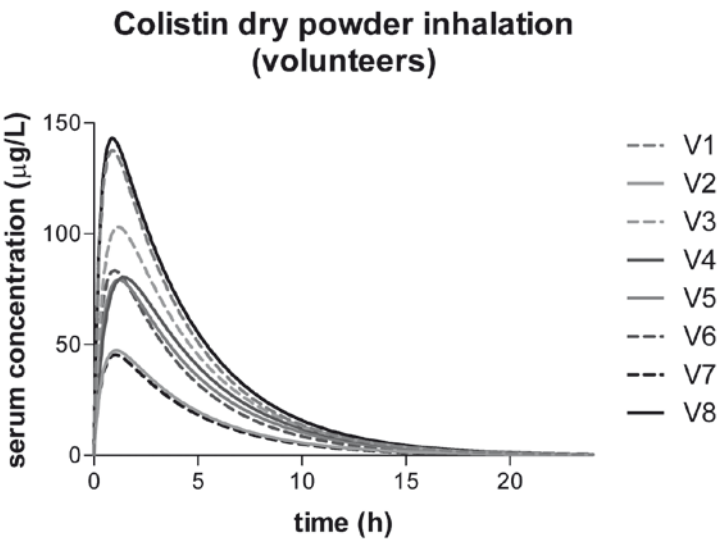

Fig. 1. Colistin dry powder inhalation (volunteers)

Table 3 Colistimethate sodium dry powder inhalation in volunteers: pharmacokinetic results

	mean	95% C.I.
Actual dose (mg)	21.5	18.6-24.4
AUC_{0-4} (h.µg/L)	274.8	185.2-364.3
C_{max} (µg/L)	89.9	59.4-120.4
t_{max} (h)	1.1	0.9-1.2
Ka (h^{-1})	2.4	1.9-2.8
$t_{1/2,el}$ (h)	2.75	2.68-2.82
Cl/F (L/h/kg)	51.2	36.3-66.0

Actual dose: nominal dose minus remainder of colistimethate sodium in inhaler after inhalation. AUC_{0-4}: area under the curve from 0 to 4 h. *C*max: maximum plasma concentration. t_{max}: time to maximum plasma concentration. $t_{1/2,el}$: terminal half-life. Cl/F: clearance following pulmonary administration; F: bioavailability (unknown). C.I.: confidence interval.

Discussion

This pilot study was intended to investigate the feasibility, including tolerability and potential clinical use, of a single dose of a dry powder formulation of colistimethate sodium in a newly developed inhaler in healthy volunteers. The results are promising and justify a subsequent study in cystic fibrosis patients.

To our knowledge, no study on dry powder inhalation of colistimethate sodium in healthy volunteers has been published before. Le Brun *et al.,* (2002) investigated a dry powder formulation of colistin sulfate, using an inhaler prototype based on single classifier technology. Colistimethate sodium, used in the present study, is converted in vivo into a large number of derivatives, of which a substantial amount is turned into colistin and colistin sulfate. It has been shown that colistin sulfate shows a stronger antimicrobial effect than the parent compound (Schwartz *et al.,* 1959-1960; Barnett *et al.,* 1964; Beveridge and Martin 1967). Volunteers and patients experienced cough as adverse effect of inhalation of colistin sulfate, but the dry powder device was appreciated by the patients despite these adverse effects. In a subsequent study, Westerman *et al.* (2004) found that the sulfate salt of colistin was responsible for these adverse effects. This resulted in the use of the sulfomethate salt of colistin in the current study. The colistimethate sodium dry powder inhalation was well tolerated by all volunteers. No clinically relevant influence on lung function was measured, nor did any of the volunteers experience adverse effects during or after inhalation.

Pharmacokinetic profiles, based on colistin serum concentrations, give insight into pulmonary deposition of the drug and can be used for comparison of different methods of inhalation of the same drug (Auty *et al.,* 1987; Lipworth 1996). Serum concentration data of colistimethate sodium and derived pharmacokinetic parameters in the current study should be compared cautiously with data from Le Brun *et al.,* (2002), retrieved from a different dry powder inhaler and from both colistin sulfate (DPI) and colistimethate sodium (nebulisation). Comparison of

our values with pharmacokinetic data from intravenously used colistin (Reed *et al.,* 2001; Li *et al.,* 2003) is difficult because of the different methods of serum sample analysis and pharmacokinetic analysis used.

Using serum concentrations as surrogate parameters of lung deposition implies that no drug should be absorbed in the gastro-intestinal tract. It is generally accepted that colistin is not absorbed after oral administration. However, no supporting data in literature were known to us. To determine if gastro-intestinal absorption of colistimethate sodium, expressed in serum concentration, occurs, we subjected the volunteers to an oral intake of colistimethate sodium. The results confirm that oral intake of colistin indeed does not result in a significant contribution to serum concentration.

Successful drug inhalation depends on the individual patient and specifications of the drug and inhaler device (Brand *et al.,* 2000). In general, an aerodynamic particle size of 1–5 µm is required to obtain peripheral pulmonary deposition. In dry powder inhalation, a proper balancing between the obtained particle size distribution in the aerosol and the inspiratory flow rate is of main importance. The Twincer® inhaler has been subjected to extensive *in vitro* testing in combination with an optimised formulation of colistimethate sodium. This resulted in a relatively simple inhaler design intended for single use that is low in production costs, and a micronised colistin dry powder formulation that, with the use of lactose sweeper crystals, can be administered with high efficiency due to the properties of the inhaler (De Boer *et al.*, 2006).

To prevent small particles from being exhaled and to improve pulmonary deposition (by sedimentation and diffusion), the participants in this study were instructed to perform a deep and powerful inhalation, lasting about 2–3 s, and subsequently to hold their breath for a few seconds. This instruction was based upon *in vitro* results obtained with the same the dry powder formulation and inhaler device (De Boer *et al.*, 2006). The maximal inspiratory flow rates generated by our subjects and the inhalation times were within the predefined range. The volunteers inhaled the study drug immediately after instruction with an empty inhaler in order to be able to compare the measured maximum inspiratory flow rate and inhalation time to those achieved during inhalation of the drug.

The dose was calculated based upon the *in vitro* efficiency of the inhaler: at a constant inspiratory flow rate of 67 l/min (4 kPa) approximately 60% of the powder mixture is in size range of 1–5 µm (fine particle fraction, De Boer *et al.*, 2006). Subsequently, the dose was adjusted to an average pulmonary deposition of 10% reached after nebulisation of a 160 mg drug dose with a Ventstream® nebuliser and a PortaNeb® compressor (Le Brun *et al.,* 1999). The amount of colistimethate sodium lost during inhalation due to inhaler retention (approximately 7%) in our study is in line with the *in vitro* experiments by De Boer *et al.* (2006).

In conclusion, dry powder inhalation of 25 mg colistimethate sodium, using the newly developed Twincer® inhaler, is well tolerated by healthy volunteers. A subsequent pilot study in cystic fibrosis patients is therefore considered to be safe in developing a dry powder inhalation of colistin for everyday treatment of patients with CF.

Acknowledgements

The authors wish to thank Mr. P. Hagedoorn (Department of Pharmaceutical Technology and Biopharmacy, University of Groningen) for his technical assistance in preparing the study, Mr. G. van der Meyden (Adult Cystic Fibrosis Center, Haga Teaching Hospital) for performing the pulmonary function tests and Mr. H. Trumpie (Clinical, Pharmaceutical and Toxicological Laboratory, Apotheek Haagse Ziekenhuizen) for the optimisation of the colistin analysis and assay of the serum concentrations.

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